

A new type of Kranz anatomy in Asteraceae

Guadalupe Peter^{A,C} and Liliana Katinas^B

^ADepartamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur,
San Juan 670 (8000) Bahía Blanca, Argentina.

^BDivisión Plantas Vasculares, Museo de La Plata, Paseo del Bosque s/n (1900) La Plata, Argentina.

^CCorresponding author; email: gpeter@criba.edu.ar

Abstract. The anatomical structure of the leaves and stems of the 13 species of *Isostigma* (Asteraceae: Heliantheae) has been examined by using light microscopy. All species of *Isostigma* have Kranz anatomy in their leaves, containing one or more Kranz units (=KU, the unit constituted by the vascular bundle/s, the parenchyma sheath and the surrounding mesophyll). It is demonstrated that there are the following two different types of Kranz anatomy in leaves of *Isostigma*: (1) *Eryngiophyllum* type, with one KU per leaf and with sclerenchyma tissue (*I. brasiliense*, *I. cordobense*, *I. crithmifolium*, *I. dissitifolium*, *I. peucedanifolium*, *I. riedelii*, *I. simplicifolium* and *I. speciosum*); and (2) *Isostigma* type, with more than one KU per leaf, without sclerenchyma tissue (*I. acaule*, *I. herzogii*, *I. hoffmannii*, *I. molfinianum* and *I. scorzoneraefolium*). The stems of all 13 species of *Isostigma* show also Kranz anatomy, without variation among species. Until the present, the *Eryngiophyllum* and the *Atriplicoid* types were the only reported for Asteraceae. The *Isostigma* type is a new type for the family, characteristic of plants growing in humid places. The following evolutionary sequence of Kranz anatomy is hypothesised: *Atriplicoid*–*Isostigma*–*Eryngiophyllum* where numerous KUs become continuous to reach a unique, compound KU.

Introduction

The C₄ photosynthetic syndrome consists of anatomical, physiological and biochemical components, all of which must be present to facilitate proper functioning of the CO₂-concentrating mechanism that C₄ plants possess in addition to the Calvin–Benson (C₃) pathway of carbon fixation (Björkman 1973; Fisher *et al.* 1997).

The Kranz anatomy is intimately linked to the C₄ photosynthesis. Haberlandt (1882, 1914) used the term Kranz to refer to the wreath of radially arranged mesophyll cells surrounding the leaf bundle sheath. Currently, the term is applied to both the enlarged chloroplast-rich bundle-sheath cells (Kranz cells) and to the entire suite of distinctive structural characteristics (Kranz anatomy) (Dengler and Nelson 1999). Variation in Kranz anatomy in genera and species led to the establishment of different Kranz types as shown for example in the classifications of Johnson and Brown (1973), Brown (1975), Carolin *et al.* (1975) and Pyankov *et al.* (2001).

Knowledge of the presence or absence of Kranz anatomy has been recognised of taxonomic, phylogenetic, ecological and economic interest in plant families and genera. Therefore, the different aspects of this photosynthetic pathway were extensively analysed, focusing principally on Poaceae (e.g. Johnson and Brown 1973; Zuloaga *et al.* 1989) within the Monocotyledonae and Chenopodiaceae (e.g.

Carolin *et al.* 1975, 1978) within the Dicotyledonae. However, these types of studies in Asteraceae are not extensively developed. Some representative work has been done by Schöch (1971), Brown (1975), Smith and Turner (1975), Sánchez *et al.* (1986, 1987), Moore *et al.* (1987), Sarmiento *et al.* (1989, 1995), Araus *et al.* (1990), Petenatti and Del Vitto (2000) and Peter (2001), with only a few of them dealing with anatomy.

Species of Asteraceae that possess the high ¹³C : ¹²C ratio typical of Kranz syndrome were found in only two tribes, Heliantheae and Helenieae (Smith and Turner 1975), with 150 species in five genera (2% of all C₄ and 1.3% in the family) (Sage *et al.* 1999). In the Helenieae only two genera, *Flaveria* and *Pectis*, have C₄ anatomy. In the Heliantheae, one species of *Parthenium* was found C₃–C₄ intermediate (Moore *et al.* 1987), whereas C₄ syndrome is exclusive of three genera belonging to the subtribe Coreopsidinae, namely *Chrysanthellum* (= *Eryngiophyllum*; 13 species from Mexico to South America, Galapagos, Africa and India), *Isostigma* (c. 13 South American species) and *Glossocardia* (= *Glossogyne*, *Guerreroia*, *Neuractis*; 12 species from Asia and Australia) (Smith and Turner 1975; Kellogg 1999; Sage *et al.* 1999). These three genera, with the addition of *Diodontium* and *Trioncinia* (both genera of Australia), are recognised as the *Chrysanthellum* group (Karis and Ryding 1994).

Prior to this study, the only published C_4 anatomical studies of some of the above mentioned genera were performed by [Brown \(1975\)](#) and [Sánchez *et al.* \(1986, 1987\)](#). [Brown \(1975\)](#) established the *Eryngiophyllum* type for genera of Chenopodiaceae and Asteraceae, characterised by continuous single layers of mesophyll and parenchyma sheath beneath the epidermis. Almost at the same time, [Carolin *et al.* \(1975\)](#) assigned other new type names for the same genera of Chenopodiaceae considered by [Brown \(1975\)](#). The genera of Asteraceae examined by [Brown \(1975\)](#) were *Chrysanthellum*, *Eryngiophyllum* (currently *Chrysanthellum*), *Flaveria*, *Glossocardia*, *Glossogyne* (currently *Glossocardia*), *Isostigma* and *Pectis*. In his study, [Brown \(1975\)](#) did not specify which species of each genus were analysed. Furthermore, [Sánchez *et al.* \(1986\)](#) found another C_4 anatomy in the leaves of *Chrysanthellum argentinum* and *C. tuberculatum* (considered synonyms of *C. indicum* DC. var. *afroamericanum* B.L. Turner by [Turner 1988](#)), without mentioning or establishing a Kranz type for them. Both species show the typical parenchyma sheath of cells with large chloroplasts and a mesophyll layer surrounding each vascular bundle. In addition, Kranz structure in stems of both species of *Chrysanthellum* was found ([Sánchez *et al.* 1987](#)). It is interesting to note that there is not a strict correlation between the presence of Kranz anatomy in the leaves and stems, since for example species of the genus *Pectis* (Asteraceae: Helenieae) possess Kranz anatomy exclusively in leaves ([Sánchez *et al.* 1987](#)).

The South American genus *Isostigma*, considered the most closely related genus to *Chrysanthellum* ([Ryding and Bremer 1992](#); [Karis and Ryding 1994](#)), is a little-studied assemblage of *c.* 13 species ([Sherff 1926](#); [Sherff 1931](#); [Cabrera 1959](#)) of perennial herbs and subshrubs of north-eastern and central Argentina, southern and eastern Brazil, south-eastern Bolivia, Paraguay and Uruguay. Some species are known from the type collection only; many are quite localised, occurring only in the mountain ranges of central Argentina. With relatively few species, *Isostigma* grows in different habitats such as dry, subhumid and humid places. A revision of the genus *Isostigma* in progress ([G. Peter, unpubl. data](#)), led to focus our attention to the particular structure of leaves. Although [Brown \(1975\)](#) reported the *Eryngiophyllum* type for leaves of this genus, we noted variation among species in their Kranz anatomy. The main objective of the present study was to investigate this variation.

Material and methods

Leaves and stems of the 13 species of *Isostigma* were obtained from herbarium specimens. Localities and vouchers are listed in the Appendix. Material of most species was submerged in ethyleneglycol for 6 days ([Schwabe 1961](#)), then fixed in FAA (formalin–acetic acid–alcohol) and processed by usual techniques of paraffin infiltration. Serial transverse sections were cut 10 μ m thick with a Minot microtome and stained with Safranin for 8 h and Fast Green for

25 s; sections were mounted in Canada balsam. Free-hand sections of leaves and stems of all species were also cut, some sections were cleared and stained with Safranin while the rest were left unstained and mounted in glycerin jelly. Whole leaves and leaflets were cleared according to [Strittmatter \(1973\)](#).

Light microscopy observations were carried out on a Leitz SM Lux with camera lucida technique. Selected light microscopy images were transferred electronically from the microscope to the computer using the Photo Express version 1.0 software.

Some terms employed in the Results and Discussion are here defined:

Kranz unit (KU): is constituted by the vascular bundle/s enclosed in the parenchyma sheath and the surrounding mesophyll. [Sánchez *et al.* \(1986\)](#) referred to these units as ‘unidades histofoliarias’. KUs can be simple if they have only one vascular bundle, or they can be compound if the KUs have more than one vascular bundle.

Vein connection: the longitudinal veins are interconnected by oblique or transversal, short veins. The mesophyll and the parenchyma sheath of the KU can accompany the vascular tissue in its connection.

Vein branching: the longitudinal veins are laterally diverging in short veins, but they do not connect with neighbour veins and end in the mesophyll.

Results

In all the specimens examined the leaves and stems show some common features. The mesophyll consists of a single layer of cylindrical, radially enlarged, cells that are densely packed, with chloroplasts commonly located peripherally, along the cell walls. The parenchyma sheath is a single layer of cubical cells with large chloroplasts, with respect to those of the mesophyll; chloroplasts are positioned against the internal tangential cell walls ([Fig. 1a](#)). The colourless parenchyma is constituted by translucent, large and isodiametric cells, with thin walls; there are few or no chloroplasts.

Leaves

Leaves of *Isostigma* are entire, pinnati- or bipinnatisect. The entire leaves can be linear to narrowly ovate with the apex entire (*I. herzogii*, *I. scorzoneraefolium*) or briefly bi-tridentate (*I. molfinianum*), obovate with the apex dentate (*I. acaule*), or linear, very long with the apex entire or tridentate (*I. riedelii*, *I. simplicifolium*). The pinnati- or bipinnatisect leaves have linear (*I. crithmifolium*, *I. dissitifolium*, *I. speciosum*), filiform (*I. brasiliense*, *I. cordobense*, *I. peucedanifolium*) or ovate (*I. hoffmannii*) leaflets.

All species have amphistomatic leaves. The venation patterns are variable in *Isostigma*. In *I. acaule* and *I. hoffmannii* the veins are reticulate. In *I. brasiliense*, *I. cordobense*, *I. crithmifolium*, *I. dissitifolium*, *I. peucedanifolium*, *I. riedelii*, *I. simplicifolium* and *I. speciosum* the pattern in major and minor veins is parallel. In *I. herzogii*, *I. molfinianum* and *I. scorzoneraefolium* the major veins are parallel with only a few connections and branches, but they have smaller veins profusely connected and branched among them ([Fig. 4b](#)).

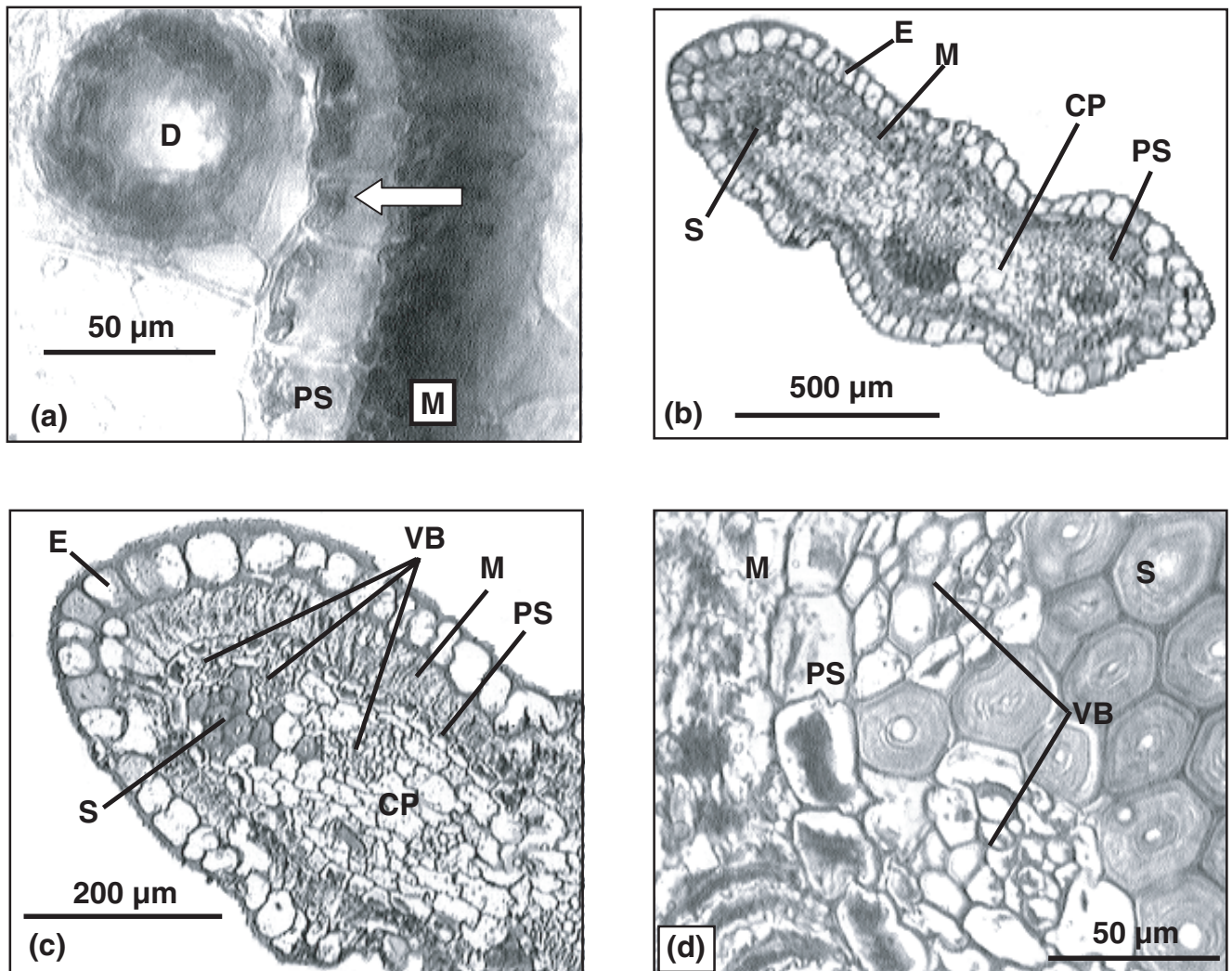


Fig. 1. Light micrographs of cross-section in leaves showing the *Eryngiophyllum* type: (a) parenchyma sheath with the chloroplasts against the internal tangential cell walls (arrow), note the duct close to the sheath; (b) cross-section of a leaflet showing the epidermis and one KU with the 1-layered mesophyll and parenchyma sheath enclosing several vascular bundles and the internal colourless parenchyma; (c) detail of the leaf margin showing the leaf tissue arrangement; (d) vascular tissue in contact with the parenchyma sheath, note the strands of xylem at both sides of the sclerenchyma. (a) *Isostigma speciosum*, Dusén 4345 (BM); (b–d) *Isostigma crithmifolium*, Grassini 340 (BBB). CP = colourless parenchyma, D = duct, E = epidermis, M = mesophyll, PS = parenchyma sheath, S = sclerenchyma, VB = vascular bundle.

All species of *Isostigma* have Kranz anatomy in their leaves, which can be grouped into two different types: (1) *Eryngiophyllum* type and (2) *Isostigma* type. The *Eryngiophyllum* type was established by Brown (1975) and the *Isostigma* type is established here.

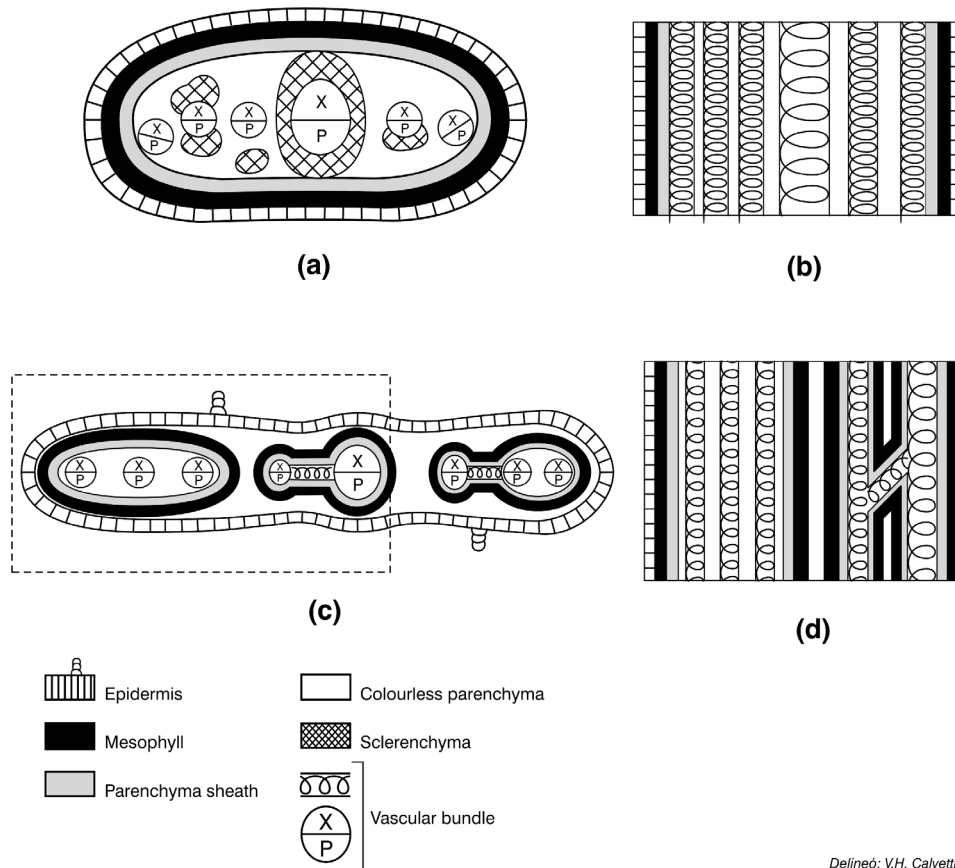
Eryngiophyllum type (Brown 1975) (Figs 1, 2a, b)

One Kranz unit per leaf; the KU is compound; sclerenchyma present; veins are not connected (or only one or two connections per leaf) and not branched.

Transection. The KU encloses three to 11 vascular bundles (Fig. 1b). The photosynthetic mesophyll and the parenchyma sheath are continuous or rarely discontinuous

subepidermal layers. The parenchyma sheath is just internal to the mesophyll and in contact with it (Fig. 1c). There is one major, central collateral vascular bundle and one to five lateral, smaller vascular bundles that lie in the colourless internal parenchyma. Bundles of vascular tissue are often in contact with the parenchyma sheath (Fig. 1d). Secretory ducts are also present in the central parenchyma, generally associated to the vascular tissue (Fig. 1a). Sclerenchyma tissue constitutes a continuous sheath around the vascular bundles, or it forms groups near the xylem and the phloem, adjacent to the phloem, or within the colourless parenchyma.

Paradermal. Major vascular bundles run parallel and are embedded in the colourless parenchyma; veins are not



Delineó: V.H. Calvetti

Fig. 2. Diagram showing (a, b) the *Eryngiophyllum* type; (c, d) the *Isostigma* type. (a, c) Transsection, (b, d) paradermal. P = phloem; X = xylem; secretory ducts have not been drawn.

branched and lateral connections are generally lacking; when present they are very scarce (only one or two per leaf). Colourless parenchyma is limited by the parenchyma sheath at both sides (Fig. 2b).

The *Eryngiophyllum* type is present in *Isostigma brasiliense*, *I. cordobense*, *I. crithmifolium*, *I. dissitifolium*, *I. peucedanifolium*, *I. riedelii*, *I. simplicifolium* and *I. speciosum*. Most of these species have continuous layers of both mesophyll and parenchyma sheath, sometimes they are discontinuous at the margins of the leaf and abaxially at the level of the midvein (e.g. some specimens of *I. speciosum*).

Isostigma type (Figs 2c, d and 3a–e)

More than one KU per leaf; KUs are simple and compound; without sclerenchyma; veins are usually connected and branched.

Transsection. This anatomy type has 3–27 KUs per leaf (Fig. 3a). Each KU encloses one to four collateral vascular bundles associated with secretory ducts. Simple and

compound KUs coexist in the same transsection. Sometimes numerous ducts (c. 12) constitute a ring around the midvein.

Neighbour KUs are commonly connected by vascular tissue, which is accompanied by the mesophyll and the parenchyma sheath (Fig. 3b–d).

Layers of both mesophyll and parenchyma sheath surround completely the KUs, although in occasions they are interrupted at the abaxial side of the midvein. Some KUs are delimited only by the parenchyma sheath and the mesophyll surrounds two or more KUs (Fig. 3e). Colourless parenchyma is distributed beneath the epidermis and between the KUs when the KUs are simple, inside the KUs when they are compound, or adjacent to the abaxial epidermis. Sclerenchyma tissue is completely absent in this type.

Paradermal. One to four vascular bundles are limited by the parenchyma sheath and mesophyll at both sides. Veins are connected and branched (Fig. 2d).

This type is present in *Isostigma acaule*, *I. herzogii*, *I. hoffmannii*, *I. molfinianum* and *I. scorzoneraefolium*.

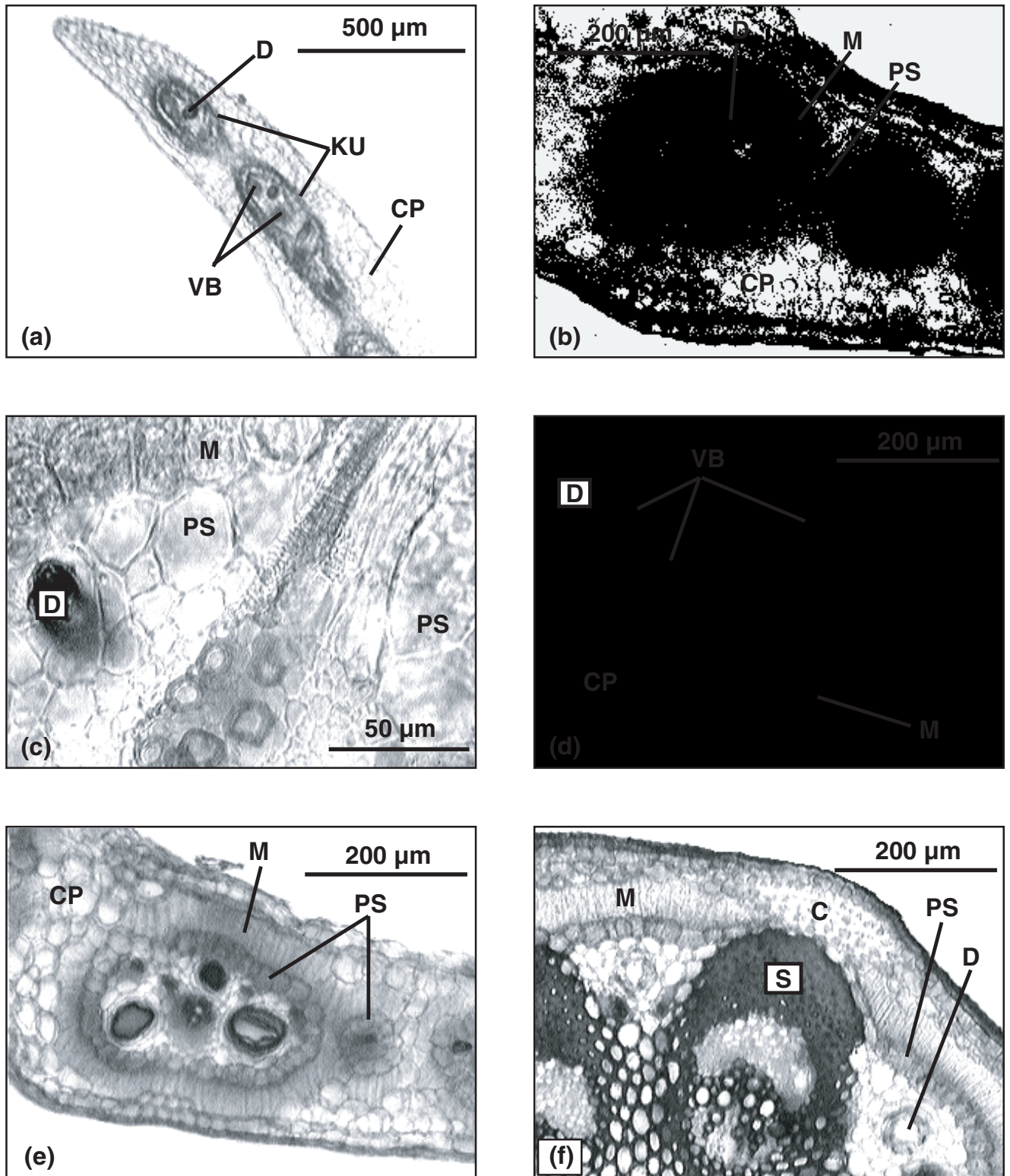


Fig. 3. (a–e) Light micrographs showing the *Isostigma* type: (a–c) cross-sections in leaves; (a) detail of the leaf margin showing two KUs enclosing several vascular bundles and secretory ducts, the colourless parenchyma surrounds the KUs; note the KU at the right connecting with a third KU; (b) vascular connection between two KUs with its accompanying mesophyll and parenchyma sheath; (c) closer view of the vascular connection; (d) paradermal view of the vascular connections showing the vascular tissue and the mesophyll; the parenchyma sheath can not be seen due to the clearing process; (e) cross-section of leaf showing adjacent veins sharing the mesophyll layer; (f) light micrograph of cross-sections in stem. (a) *I. scorzoneraefolium*, Malme 3136 (GH); (b, c) *I. hoffmannii*, Nee 49046 (NY); (d) *I. hoffmannii*, Ybarrola 2634 (LP); (e) *I. herzogii*, Herzog 617 (G); (f) *I. dissitifolium*, Hassler 6309 (F). C = collenchyma; CP = colourless parenchyma; D = duct; KU = Kranz unit; M = mesophyll; PS = parenchyma sheath; S = sclerenchyma; VB = vascular bundle.

In *I. herzogii*, *I. molfinianum* and *I. scorzoneraefolium* the amount of connections and branching of veins is variable; in some specimens they are very scarce but in others they are quite abundant. In *I. acaule* and *I. hoffmannii* most of the KUs are simple and the compound ones are in the margins.

Stem

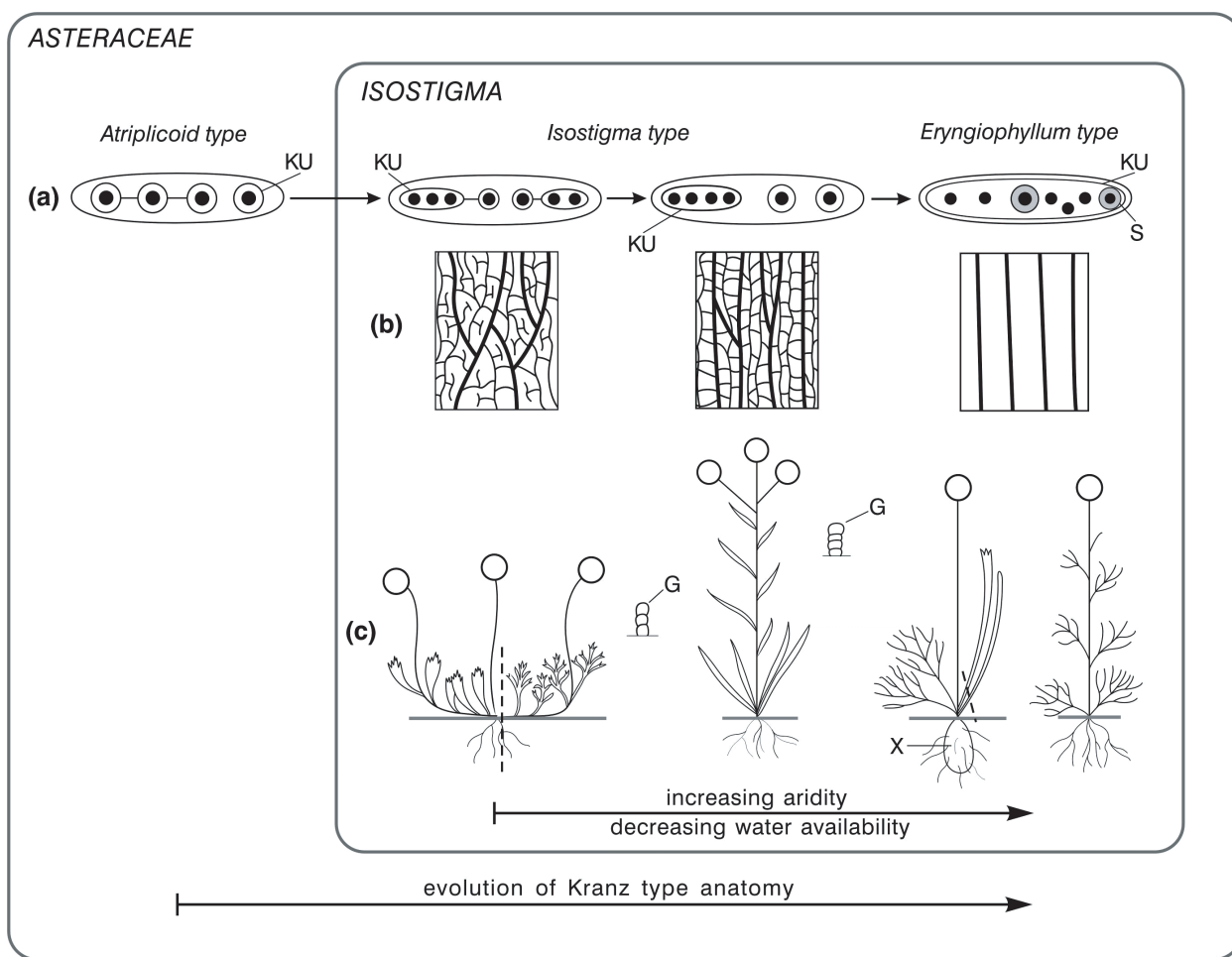
All species of *Isostigma* have Kranz anatomy in their stems, without variation among them.

There are 9–13 collateral vascular bundles bordered by sclerenchyma fibers; each bundle alternates with one secretory duct associated with minor vascular bundles. The

major vascular bundles are surrounded by sclerenchyma. Half of them are externally in contact with the ring of parenchyma sheath, followed by the mesophyll. Both parenchyma and mesophyll are single layers that become discontinuous where the subepidermal collenchyma reaches the other half of major vascular bundles (Fig. 3f).

Discussion

Our findings show that all the species of *Isostigma* have consistently Kranz anatomy in leaves and stems. The genus *Isostigma* has developed two different C_4 leaf anatomies, distinguished mainly by the number and type of Kranz units



Delineo: V.H. Calvetti

Fig. 4. Hypothesis on the evolution of Kranz anatomy in Asteraceae and in the genus *Isostigma* associated with other plant features. (a) The *Atriplicoid* type (e.g. *Chrysanthellum indicum* var. *afroamericanum*) with numerous and simple KUs; the *Isostigma* type with numerous, simple and compound KUs is an intermediate step and the *Eryngiophyllum* type with only one compound KU and sclerenchyma tissue; (b) change in the patterns of vascularisation from major veins (thick lines) connected and branched (on the left); major veins parallel, with few connections and branches (in the centre); parallel veins scarcely connected and branched (on the right); (c) different habits in *Isostigma* from hygrophyllous decumbent species with basal, alternate leaves and solitary capitula (on the left); xeric and hygrophyllous species with leaves distributed along the stem and capitula in synflorescences (in the centre). Both groups of species belong to the *Isostigma* type and possess glandular hairs. Xeric species belonging to the *Eryngiophyllum* type, generally with xylopodium, rosulate or distributed leaves without glandular hairs and solitary capitula (on the right). G = glandular hair; KU = Kranz unit; S = sclerenchyma; X = xylopodium; the dotted lines represent variation in leaf shapes.

(KUs) per leaf that range from one compound KU (*Eryngiophyllum* type) to several KUs, enclosing one to four veins (*Isostigma* type).

Kranz types in Asteraceae

The *Eryngiophyllum* type has been the only type described exclusively for Asteraceae (Brown 1975), while the *Atriplicoid* type of Chenopodiaceae has been reported for species of *Flaveria* and *Parthenium* (Pyankov *et al.* 2001). According to the leaf anatomy described by Sánchez *et al.* (1986) and our own observations, *Chrysanthellum indicum* var. *afroamericanum* also corresponds to the *Atriplicoid* type characterised by a single parenchyma sheath surrounding each vein and the mesophyll arranged radially in relation to the veins. Therefore, the *Isostigma* type is a new type for Asteraceae.

If we compare the Kranz types of Asteraceae with those of other dicot or monocot families, the *Eryngiophyllum* type resembles the *Kranz-Suaedoid* of Chenopodiaceae (Carolin *et al.* 1975), except that the parenchyma sheath in the former is more closely associated with the veins (Fig. 1d) and the colourless parenchyma replaces the water storage tissue typical of most Chenopodiaceae (Fig. 1b). There was not found a parallelism between the *Isostigma* type and other types of C₄ anatomy in dicots or monocots.

The *Eryngiophyllum* type is the most common of the Kranz anatomies in *Isostigma*, being present in eight of the 13 species. The *Isostigma* type, on the other hand, occurs in the remaining five species.

In summary, the types of Kranz anatomy established until the present in the Asteraceae are (i) the *Eryngiophyllum* type in *Chrysanthellum*, *Flaveria*, *Glossocardia*, *Isostigma* and *Pectis*, (ii) the *Isostigma* type in *Isostigma* and (iii) the *Atriplicoid* type in *Chrysanthellum*, *Flaveria* and *Parthenium*.

Kranz types and ecology

Variation in C₄ types in relation to ecological patterns was predicted by some authors, e.g. Welkie and Caldwell (1970), Hattersley (1976) and Brown (1977). Accordingly, the two different Kranz anatomies found in *Isostigma* can be correlated with environmental conditions together with some other plant features (Fig. 4).

The species of *Isostigma* inhabit six biogeographic provinces in South America, according to the classification of Cabrera and Willink (1980) (Table 1). Table 1 shows that in general the *Isostigma* type corresponds to species growing in moist places, whereas the *Eryngiophyllum* type is characteristic of xeric places.

Species of *Isostigma* with the *Eryngiophyllum* type are mostly erect, glabrous herbs or subshrubs with solitary capitula and mostly rosulate leaves, that grow under high-light, hot and mostly arid conditions. Most of these species have xylopodium, a tuberous root for water storage (Font Quer 1979). This structure and the centric anatomy in leaves are mechanisms of water-use efficiency, while foliar sclerenchyma prevents tissue damage caused by desiccation (Metcalf 1983). The close proximity of the continuous mesophyll cell layer to the atmosphere ensures the effective

Table 1. Kranz anatomy, species of *Isostigma* corresponding to each type, biogeographic provinces and type and characteristics of habitat taken from the literature and label data

Biogeographic provinces correspond to Cabrera and Willink (1980)

Species	Biogeographic province	Type of habitat	Habitat characteristics
<i>Isostigma</i> type			
<i>I. acaule</i>	Chacoan–Parana	Humid	Parque, swamp, low and modified places; clay soil
<i>I. herzogii</i>	Chacoan	?	High field (600 m)
<i>I. hoffmannii</i>	Chacoan–Espinal	Humid	Pampa, savanna, grassland, open forest; clay or sandy soil but waterlogged in some seasons
<i>I. molfinianum</i>	Prepunan	Humid	Marshy places
<i>I. scorzoneraefolium</i>	Cerrado	Subhumid–xeric	Open field, grassland, subhumid places; sandy-gravelly soil
<i>Eryngiophyllum</i> type			
<i>I. brasiliense</i>	Cerrado	Subhumid?–xeric	Forest clearing, grave beach; sandy soil
<i>I. cordobense</i>	Chacoan	Xeric	Low hill
<i>I. crithmifolium</i>	Parana–Pampean–Espinal	Xeric	Savanna, dune, gravelly hill, field, sandy, dry and hilly grassland
<i>I. dissitifolium</i>	Parana–Chacoan	Xeric	Inculting hill, grassland; sandy and gravelly soil
<i>I. peucedanifolium</i>	Pampean–Parana–Cerrado–Chacoan	Subhumid?–xeric	Open and high field, sunny slope, gravelly hill, cerrado, gallery forest, grassland, savanna, fixed dunes, palm grove, ravine; sandy, clay, gravelly and dry soil
<i>I. riedelii</i>	Parana	Subhumid–xeric	Cerrado scrub and cerrado forest, dry and subhumid field, slopes and hill near river; sandy soil
<i>I. simplicifolium</i>	Cerrado	Xeric	Cerrado scrub and cerrado forest, field; sandy soil
<i>I. speciosum</i>	Cerrado–Parana	Xeric	Dry field, cerrado forest and cerrado scrub

assimilation of atmospheric CO₂ (Ehleringer and Monson 1993) and high-light use. The colourless parenchyma in the leaf center can be seen as another water-storage system of the plants and it is important in the hydric economy.

Species with the *Isostigma* type of Kranz anatomy have sparse glandular hairs, lack sclerenchyma tissue completely and grow mostly in humid places. The presence of more than one KU per leaf in the *Isostigma* type led to a re-arrangement of the leaf tissues when compared with the *Eryngiophyllum* type. The mesophyll and the parenchyma sheaths are not continuous and are not completely adjacent to the epidermis. The colourless parenchyma surrounds the KUs or, less commonly, lies inside the major KUs. This changed tissue arrangement should not be as efficient in light and water use as that of *Eryngiophyllum* type, probably because the habitat conditions are not so harsh. The lack of sclerenchyma also suggests that the species with this type of Kranz anatomy are not so exposed to periods of desiccation.

Within the *Isostigma* type, some species (*I. acaule*, *I. hoffmannii*) are decumbent herbs inhabiting humid places, with solitary capitula and leaves present only at the point of contact between the stem and the soil, otherwise the stems are sparsely bracteolate (Fig. 4). All the specimens examined of these two species showed abundant vein branching and connection. Other species (*I. herzogii*, *I. molfinianum*, *I. scorzoneraefolium*) are erect herbs or shrubs with corymbose synflorescences and leaves spreading along the stems and grow in humid to xeric habitats. Some of the examined specimens showed scarce vascular connections and branching in their major veins. These differences in the venation pattern in species with *Isostigma* type would suggest a correlation between an increase in vein branching and connection and humid habitats. This correlation has been reported in other C₄ genera such as *Echinochloa* (Poaceae) (A. M. Arambarri, pers. comm.).

Kranz types and evolution

Framing the different leaf Kranz anatomies analysed here in an evolutionary scenario, it can be hypothesised that species of *Isostigma* changed the use of a 'hose' to a 'water tank' or, in other words, the replacement of vascular connections in species of humid and mesic places by central colourless parenchyma to increase the water-use efficiency in species of arid places (Fig. 4). The correlation between the specialisation in leaf anatomy and the habitat of limited water availability was already pointed out by Welkie and Caldwell (1970) for other dicot families with C₄ species.

According to Brown (1975), the continuous parenchyma sheath of the *Eryngiophyllum* type may have evolved by all adjacent bundle sheaths becoming continuous by lateral bundle sheath extensions (similar to those of the *Isostigma* type) followed by filling the interior with massive colourless parenchyma. The *Atriplicoid* type, present in some genera of Asteraceae, with its numerous, simple KUs, could be the

starting point of such sequence, the numerous, simple and compound KUs of the *Isostigma* type being the intermediate stepping stone, to reach the unique, compound KU of the *Eryngiophyllum* type.

The following three main lines of evidence led us to suggest a sequence from the *Atriplicoid* to the *Eryngiophyllum* type: (1) ontogeny; (2) similar processes in other plant families; and (3) historical biogeography. First, it was postulated (Brown 1975; Soros and Dengler 2001) that the parenchyma sheaths originate from the same procambium that develops into the vascular bundles. Following this, the evolutionary sequence in Asteraceae could start from the *Atriplicoid* type, with various independent parenchyma sheaths surrounding each vascular bundle and then fusing (*Isostigma* type) to end in the *Eryngiophyllum* type with its unique, continuous layer enclosing all the bundles. The opposite sequence, i.e. from the *Eryngiophyllum* to the *Atriplicoid* type, would be more complex, since it would involve various sheaths fusing into a unique layer and then splitting again into various sheaths to surround each bundle. Second, other plant families such as Chenopodiaceae seem to have followed a similar sequence (Carolin *et al.* 1975) where various, independent arcs of partial parenchyma sheath and mesophyll (*Kochioid* type) derived into complete layers of Kranz cells and mesophyll surrounding the vascular bundles (*Salsoloid* type). Third, there was a change from humid to xeric environments in the climatic history of southern South America at the time of the early evolution of Asteraceae. These changes greatly affected the biota of the area, which had to adjust to the arid conditions.

It was hypothesised that occurrence of C₄ photosynthesis in Dicotyledonae appeared recently in geological time, possibly in response to the depleted atmospheric CO₂ levels of the late Miocene and the Quaternary (Ehleringer *et al.* 1997). During the Miocene (25–5 million years ago), floristic assemblages analogous to the actual types of subtropical and temperate forest rose in southern South America (Villagrán and Hinojosa 1997), with savanna–grassland vegetation covering part of north-eastern Argentina, Uruguay and southern Brazil. Miocene could be the period of appearance of taxa with the *Atriplicoid* type of Kranz anatomy. During the Pliocene (5–1 million years ago), the distribution of this flora was fragmented and xeric formations expanded. The uplift of the Andes created the extremely dry Chaco and Monte. During Pleistocene, local glaciation resulted in a series of adjustments that resulted in numerous speciation events within genera (Stuessy *et al.* 1996). Pleistocenic events in the Quaternary, with their series of humid and arid phases (Haffer 1969; Hammen 1974; Simpson 1975), were fundamental in the biotic history of South America (Katinas *et al.* 1999; Katinas and Crisci 2000). During arid phases the xeric forms were favoured for migration and establishment, and mesic forms were isolated

in refugia and evolved there. Isolation favoured the evolution of new species of the mesic forms during the dry phases. During humid phases the opposite occurred. Xeric species became isolated in dry refugia and further speciated, while the mesic species dispersed and became established in a wider range until the next stage in the cycle of climatic change. This model, proposed for the Neotropical CAM Bromeliaceae (Gilmartin 1983) could also explain the current C_4 types in species of *Isostigma* inhabiting either wet (*Isostigma* type) or dry habitats (*Eryngiophyllum* type).

In conclusion, diversity in habit, morphological adaptive features and habitat, seems to be correlated with the different Kranz types in *Isostigma*. Further studies including all the genera of the *Chrysanthellum* group in a phylogenetic framework will allow a more comprehensive evaluation of the evolution of Kranz anatomy in this complex.

Acknowledgments

We thank Ana Arambarri and Nancy Dengler for critical reading of the manuscript and two anonymous reviewers for their valuable suggestions. Curators of herbaria for loan of specimens and Victor H. Calveti for delineating Figs 2 and 4 are also acknowledged. This work was supported by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and Universidad Nacional del Sur (UNS).

References

Araus JL, Brown RH, Bouton JH, Serret MD (1990) Leaf anatomical characteristics in *Flaveria trinervia* (C_4), *Flaveria brownii* (C_4 -like) and their F_1 hybrid. *Photosynthesis research* **26**, 49–57.

Björkman O (1973) Comparative studies on photosynthesis in higher plants. *Photophysiology* **8**, 1–63.

Brown WV (1975) Variations in anatomy, associations, and origins of Kranz tissue. *American Journal of Botany* **62**, 395–402.

Brown WV (1977) The Kranz syndrome and its subtypes in grass systematic. *Memoirs of the Torrey Botanical Club* **23**, 1–97.

Cabrera AL (1959) Ocho compuestas sudamericanas nuevas. *Notas del Museo de La Plata* **19**, 202–204.

Cabrera AL, Willink A (1980) 'Biogeografía de América Latina (2nd edn).' (The general Secretariat of the Organization of American States: Washington, DC)

Carolin RC, Jacobs SWL, Vesk M (1975) Leaf structure in Chenopodiaceae. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* **95**, 226–255.

Carolin RC, Jacobs SWL, Vesk M (1978) Kranz cells and mesophyll in the Chenopodiales. *Australian Journal of Botany* **26**, 683–698.

Dengler NG, Nelson T (1999) Leaf structure and development in C_4 plants. In 'C₄ plant biology'. (Eds RF Sage, RK Monson) pp. 133–172. (Academic Press: New York, London)

Ehleringer JR, Monson RK (1993) Evolutionary and ecological aspects of photosynthetic pathway variation. *Annual Review of Ecology and Systematics* **24**, 411–439.

Ehleringer JR, Cerling TE, Helliker BR (1997) C_4 photosynthesis, atmospheric CO_2 , and climate. *Oecologia* **112**, 285–299.

Fisher DD, Jochen Schenk H, Thorsch JA, Ferren Jr WR (1997) Leaf anatomy and subgeneric affiliations of C_3 and C_4 species of *Suaeda* (Chenopodiaceae) in North America. *American Journal of Botany* **84**, 1198–1210.

Font Quer P (1979) 'Diccionario de Botánica.' (Editorial Labor: Barcelona)

Gilmartin AJ (1983) Evolution of mesic and xeric habitats in *Tillandsia* and *Vriesea* (Bromeliaceae). *Systematic Botany* **8**, 233–242.

Haberlandt G (1882) Vergleichende Anatomie des Assimilatorischen Gewebesystems der Pflanzen. *Jahrbücher für wissenschaftliche Botanik* **13**, 74–188.

Haberlandt G (1914) 'Physiological plant anatomy.' (Macmillan: London)

Haffer J (1969) Speciation in Amazonian forest birds. *Science* **165**, 131–137.

Hammen T (1974) Pleistocene changes of vegetation and climate in tropical America. *Journal of Biogeography* **1**, 3–26.

Hattersley PW (1976) 'Speciation and functional significance of the leaf anatomy of C_4 plants.' (Australian National University: Canberra)

Holmgren PK, Holmgren NH, Barnett LC (1990) 'Index herbariorum. Part 1: the herbaria of the world (8th edn).' (International Association for Plant Taxonomy by New York Botanical Garden: Bronx, NY)

Johnson SC, Brown WV (1973) Grass leaf ultrastructural variations. *American Journal of Botany* **60**, 727–735.

Karis PO, Ryding O (1994) Tribe Heliantheae. In 'Asteraceae: cladistics and classification'. (Ed. K Bremer) pp. 559–624. (Timber Press: Portland, OR)

Katinas L, Crisci JV (2000) Cladistic and biogeographic analyses of the genera *Moscharia* and *Polyachyrus* (Asteraceae, Mutiseae). *Systematic Botany* **25**, 33–46.

Katinas L, Morrone JJ, Crisci JV (1999) Track analysis reveals the composite nature of the Andean biota. *Australian Journal of Botany* **47**, 111–130.

Kellogg EA (1999) Phylogenetic aspects of the evolution of C_4 photosynthesis. In 'C₄ plant biology'. (Eds RF Sage, RK Monson) pp. 411–444. (Academic Press: New York, London)

Metcalf CR (1983) Ecological anatomy and morphology general survey. In 'Anatomy of dicotyledons (2nd edn), Vol. 2'. (Eds CR Metcalfe, L Chalk) pp. 126–156. (Clarendon Press: Oxford)

Moore BD, Franceschi VR, Cheng S, Jingrui W, Ku MSB (1987) Photosynthetic characteristics of the C_3 - C_4 intermediate *Parthenium hysterophorus*. *Plant Physiology* **85**, 984–989.

Petenatti EM, Del Vitto LA (2000) Estructura Kranz en las especies argentinas de *Flaveria* (Asteraceae–Helenieae). *Kurtziana* **28**, 251–257.

Peter G (2001) Anatomía foliar de *Isostigma crithmifolium* (Asteraceae, Heliantheae). *Boletín de la Sociedad Argentina de Botánica* **36** (Suplemento), 26.

Pyankov V, Ziegler H, Kuz'min A, Edwards G (2001) Origin and evolution of C_4 photosynthesis in the tribe Salsoleae (Chenopodiaceae) based on anatomical and biochemical types in leaves and cotyledons. *Plant Systematics and Evolution* **230**, 43–74.

Ryding O, Bremer K (1992) Phylogeny, distribution, and classification of the *Coreopsidaeae* (Asteraceae). *Systematic Botany* **17**, 649–659.

Sage RF, Li M, Monson RK (1999) The taxonomic distribution of C_4 photosynthesis. In 'C₄ plant biology'. (Eds RF Sage, RK Monson) pp. 551–584. (Academic Press: New York, London)

Sánchez E, Arriaga MO, Panarello HO (1986) El síndrome de 'Kranz' en Asteraceae de la flora argentina. *Boletín de la Sociedad Argentina de Botánica* **24**, 249–259.

Sánchez E, Arriaga MO, Panarello HO (1987) El síndrome de Kranz en tallos de *Chrysanthellum* (Asteraceae). *Boletín de la Sociedad Argentina de Botánica* **25**, 123–129.

Sarmiento MNR de, Israilev LRA de, Seeligman P (1989) El síndrome de Kranz en *Pectis sessiliflora* y *Pectis odorata* y su probable importancia como marcador filogenético y evolutivo. *Lilloa* **37**, 15–18.

- Sarmiento MNR de, Mendiondo ME, Abdala LA (1995) Síndrome de Kranz en especies de la tribu Tageteae (Asteraceae). *Lilloa* **38**, 167–168.
- Schöch E (1971) Malat und Aspartat als Hauptprodukte der CO₂-Kurzzeit Fixierung nun auch bei einer Composite. *Zeitschrift für Pflanzenphysiologie* **64**, 367–368.
- Schwabe H (1961) Glicol de etilene: un nuevo método en histología para ablandar material de herbario. *Boletín de la Sociedad Argentina de Botánica* **9**, 393–394.
- Sherff EE (1926) Revision of the genus *Isostigma* Less. *Botanical Gazette* **81**, 241–257.
- Sherff EE (1931) New or otherwise noteworthy Compositae. VI. *Botanical Gazette* **91**, 308–314.
- Simpson B (1975) Pleistocene changes in the flora of the high tropical Andes. *Palaeobiology* **1**, 273–294.
- Smith BN, Turner BL (1975) Distribution of Kranz syndrome among Asteraceae. *American Journal of Botany* **62**, 541–545.
- Soros CL, Dengler NG (2001) Ontogenetic derivation and cell differentiation in photosynthetic tissues of C₃ and C₄ Cyperaceae. *American Journal of Botany* **88**, 992–1005.
- Strittmatter CGD de (1973) Nueva técnica de diafanización. *Boletín de la Sociedad Argentina de Botánica* **15**, 126–129.
- Stuessy TF, Sang T, DeVore ML (1996) Phylogeny and biogeography of the subfamily Barnadesioideae with implications for early evolution of the Compositae. In 'Compositae: systematics, vol. 1'. Proceedings of the international Compositae conference. (Eds DJN Hind, HJ Beentje) pp. 463–490. (Royal Botanic Gardens: Kew)
- Turner BL (1988) Taxonomic study of *Chrysanthellum* (Asteraceae, Coreopsioideae). *Phytologia* **64**, 410–444.
- Villagrán C, Hinojosa LF (1997) Historia de los bosques del sur de Sudamérica, II: análisis fitogeográfico. *Revista Chilena de Historia Natural* **70**, 241–267.
- Welkie GW, Caldwell M (1970) Leaf anatomy of species in some dicotyledon families as related to the C₃ and C₄ pathways of carbon fixation. *Canadian Journal of Botany* **48**, 2135–2146.
- Zuloaga F, Morrone O, Dubcovsky J (1989) Exomorphological, anatomical, and cytological studies in *Panicum validum* (Poaceae: Panicoideae: Paniceae): its systematic position within the genus. *Systematic Botany* **14**, 220–230.

Manuscript received 26 September 2002, accepted 7 February 2003

Appendix. Collection data (country, province, locality, date of collection, collector and number and herbarium abbreviation) of the specimens analysed of *Isostigma* and *Chrysanthellum*

Names of the Herbaria are abbreviated according to Index Herbariorum (Holmgren *et al.* 1990)

- Isostigma acaule* (Baker) Chodat: Paraguay, Dept Central, near Ypané, 16.xi.1969, *Pedersen 9317* (LP); Paraguay, Dept Presidente Hayes, Jardín Botánico, xi.1939, *Rojas 8704* (LP); Paraguay, Dept Presidente Hayes, Trinidad, Parque Botánico, bañados del río Paraguay, viii.1916, *Rojas 1683b* (LP); Paraguay, in campo Tacuaral, 1897, *Hassler 3811* (BM, NY).
- I. brasiliense* (Gardner) Benth. & Hook.: Brazil, State Minas Geraes, Uberaba, 18.xii.1848, *Regnell 783* (F); Brazil, State Minas Geraes, Municipio Ithintal, Carral, 15.x.1950, *Macedo 2627* (US).
- I. cordobense* Cabrera: Argentina, Prov. Córdoba, Dept. Colón, Saldán, lomas frente al balneario San Remo, 22.xi.1964, *Ariza Espinar 1969* (CORD, LP).
- I. crithmifolium* Less.: Argentina, Prov. Corrientes, Dept. Ituzaingó, ruta 39, a 10 km de Ruta 14, 11.ii.1978, *Cabrera & Sáenz 29101* (LP); Argentina, Prov. Corrientes, Dept. not located, Río Mocoretá, 20.i.1995, *Long 664* (BBB); Argentina, Prov. Entre Ríos, Dept Colón, Parque Nacional del Palmar, roquerío frente a La Glorieta, 20.xii.1986, *Xifreda & Maldonado 600* (LP); Argentina, Prov. Entre Ríos, Dpto Federación, Costa Lago Salto Grande, campo Zampedri, Cnia. Susanche Sauce, 3.i.1999, *Grassini 340* (BBB); Argentina, Prov. Entre Ríos, Dept not located, Costa Río Uruguay, 20.xii.1961, *Gamerro 1146* (LP); Brazil, State Rio Grande do Sul, Quaraí, Estancia do Jardim, in campo, i.1945, *Rambo 26022* (LP); Paraguay, Dept. Misiones, Santiago, Estancia 'La Soledad', 27.xii.1965, *Pedersen 7682* (LP); Uruguay, Dept Paysandú, limestone country north of Quebracho, 9.xii.1943, *Bartlett 21138* (LP); Uruguay, Dept not located, Río Uruguay y arroyo Chapicuy, 21.ii.1941, *Rosengurtt B-3210* (LP).
- I. dissitifolium* Baker: Paraguay, Dept La Cordillera, Cordillera Central, Cerros de Tobatí, ix.1902, *Hassler 6309* (F); Paraguay, Dept La Cordillera, Cordillera de los Altos, xi.1902, *Fiebrig 450* (F).
- I. herzogii* Hassl.: Bolivia, Dept Santa Cruz, Santiago de Chiquitos, v.1907, *Herzog 617* (G).
- I. hoffmannii* Kuntze: Argentina, Prov. Corrientes, Dept Lavalle, ruta 12 km 753, 27.ii.1961, *Pedersen 5821* (LP); Argentina, Prov. Corrientes, Dept Mercedes, cercanías de Justino Solari, 10.iii.45, *Ybarrola 2634* (LP); Bolivia, Dept Santa Cruz, Pampa de Sta. Cruz, i.1911, *Herzog 1309* (LP); Bolivia, Dept Santa Cruz, Prov. Andrés Ibáñez, 17°55'S, 63°15'W, 18.iv.1998, *Nee 49046* (NY).
- I. molfinianum* Sherff: Argentina, Prov. Catamarca, 1.ii.1910, *Spegazzini 33306* (F); Argentina, Prov. La Rioja, Dept Chilecito, Cuesta de Catinzaco, 30.i.1906, *Kurtz 13369* (CORD); Argentina, Prov. La Rioja, Dept Rosario Vera Peñalosa, Quebrada Totoral, ii.1896, *Kurtz 9025* (LP).
- I. peucedanifolium* (Spreng.) Less.: Brazil, State Mato Grosso, Mun. Chapada dos Guimarães, Capao Seco, 15.xi.1975, *Hatschbach 37591* (LP); Brazil, State Sao Paulo, Campinas, fazenda do Campo Grande, 13.i.1940, *Lima 5312* (SP); Uruguay, Dept Artigas, Artigas, i.1936, *Chebataroff 1998* (LP).
- I. riedelii* Sch.Bip.: Paraguay, Dept Amambay-Caaguazú, Sierra de Amambay, xii.1907/1908, *Hassler 9927* (F); Paraguay, Dept Itapúa, Colonia Gral. Delgado, Estancia San Miguelito, 12.xi.1956, *Pedersen 4271* (LP); Paraguay, Dept not located, 'in regione cursus superioris fluminis Apa', xi.1901/1902, *Hassler 8047* (F).
- I. scorzoneraefolium* (Baker) Sherff: Brazil, State Mato Grosso, Cuiabá, 26.iv.1903, *Malme 3136* (GH); Brazil, State Mato Grosso, entre Morinho y Coxipó, pr. Cuiabá, 26.iv.1894, *Malme 1584* (BM).
- I. simplicifolium* Less.: Brazil, State Minas Geraes, 1840, *Claussen s/n* (BM 632486).
- I. speciosum* Less.: Brazil, State do Café, km 70 PM, 7.ii.1965, *Dombrowski 1474* (LP); Brazil, State Mato Grosso, Capao Grande, 7.iii.1904, *Dusén 4345* (BM).
- Chrysanthellum indicum* DC. var. *afroamericanum* B.L.Turner: Argentina, Prov. Córdoba, Dept. Pocho, Pampa de Pocho, 8.iv.1950, *Hunziker 8353* (LP); Argentina, Prov. Jujuy, Dept Capital, El Cucho, 21.i.1971, *Fabris 8179* (LP); Argentina, Prov. San Luis, Sas. de San Luis, El Telón, i.1907, *Baez s/n* (LP 069426).